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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
FORD, ALLISON M	

ART UNIT	PAPER NUMBER
1651	

DATE MAILED: 10/21/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/675,938	RATHJEN ET AL.	
	Examiner	Art Unit	
	Allison M Ford	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 September 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-15* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-15* is/are rejected.

7)☒ Claim(s) *2, 4, and 10* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

Applicant's election without traverse of Group I, claims 1-15, in the reply filed on 9/20/2004 is acknowledged.

Status of Application

Claims 1-15 are being examined for patentability. The Amended Claims Sheet submitted by applicant on 9/20/2004, shows claims 16-35 are cancelled; but Remarks made by applicant, submitted on 9/20/2004, states only claims 17-35 have been cancelled.

Priority

Acknowledgement is made of applicant's claim for priority to provisional application 60/414,959, filed 9/30/2002.

Information Disclosure Statement

The listing of references in the specification is not a proper information disclosure statement. 37 CFR 1.98(b) requires a list of all patents, publications, or other information submitted for consideration by the Office, and MPEP § 609 A(1) states, "the list may not be incorporated into the specification but must be submitted in a separate paper." Therefore, unless the references have been cited by the examiner on form PTO-892, they have not been considered.

Specification

The disclosure is objected to because of the following informalities: abbreviations not accompanied by complete term. For example, reference is made to 'EPL cells' in paragraph [0012]; but EPL is not defined to mean "early primitive ectoderm-like" cells until paragraph [0014]. Though all the

abbreviations are listed clearly in Table 1, it would be preferable to have the definition precede the first use of an abbreviation. Appropriate correction is requested.

Claim Objections

Claims 2 and 10 are objected to because of the following informalities: The abbreviations EPL and ES must be accompanied by the full terms they represent, "early primitive ectoderm-like" and "embryonic stem," respectively, at least the first time they appear in the claims.

Claim 4 is objected to due to a spelling error: "bane marrow-derived stem cells" should read, "bone marrow-derived stem cells."

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-15 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. See MPEP § 2136.

Applicant's claim 1 is directed to a method for directing a population of cells to differentiate along a mesodermal cell lineage, said method comprising culturing said cells in the presence of bone morphogenic protein 4 (BMP4) or a homologue, analogue or functional equivalent thereof for a time and under conditions sufficient for said cells to preferentially differentiate into mesodermal cells or cells of a mesodermal lineage. Claim 3 requires the cells to be stem cells. Claim 4 requires the stem cells to be

selected from the group consisting of embryonic stem cells, somatic stem cells, germ stem cells, adult renal stem cells, embryonic renal epithelial stem cells, embryonic endodermal stem cells, hepatocyte stem cells, mammary epithelial stem cells, bone marrow-derived stem cells, skeletal muscle stem cells, bone marrow mesenchymal stem cells, $CD34^+$ haematopoietic stem cells, and mesenchymal stem cells. Claims 2-9 have the limitation of claim 1.

Applicant describes the method by which they induced mouse embryonic stem cells to differentiate into cardiomyocytes in the presence of BMP4 (See Specification, Pg 25-27). They have not, however, provided sufficient written description to enable for any type of cell (Claim1), any type of stem cell (Claim 3), or even the types of stem cells listed in claim 4. The embryonic mouse cell is the only example given, it does not constitute a representative number of species of cells, for as one skilled in the art knows, cells differ immensely, for example terminally differentiated cells are very different then embryonic stem cells. The embryonic mouse cell is not even representative of the list of stem cells in claim 4. The types of stem cells listed in claim 4 includes what would more appropriately be called progenitor cells, they have undergone several steps of differentiation, for example, hepatocyte stem cells have undergone partial differentiation directed towards becoming hepatocytes, they do not have the same totipotency as embryonic stem cells. The different stem/progenitor cells have different receptors and different pathways; they would react differently to the same stimulus, including BMP4. For example, Finley et al teach that BMP4 only directs cells towards mesodermal lineage differentiation during a restricted time period from day 5-8 (See Finley et al, Pg 277, col. 2- Pg. 278, col.2 and Table 1); therefore an adult renal stem cell, which has partially differentiated and thus is pass day 8 of differentiation, would not have the same reaction to BMP4 as an embryonic stem cell at day 5 of differentiation. Additionally, there is no disclosure of relevant, identifying characteristics, such as the particular receptor or pathway required in the cell to be affected by BMP4, sufficient to show the applicant was in possession of the claimed genus. *See Eli Lilly,* 119F. 3d. at 1568, 43 USPQ2d at 1406.

Similarly, applicant uses bone morphogenic protein 4 (BMP4) to induce differentiation of mouse ES cells into cells of mesodermal lineage (See Specification, Pg. 25-27). However claim 1 is drawn to BMP4 or a homologue, analogue or functional equivalent thereof. There is insufficient written description provided in the disclosure to adequately describe the BMP4 used in the experiment, as they do not disclose the species of origin, therefore there is complete lack of written description to sufficiently describe homologues, analogues, and functional equivalents thereof. It is understood that mouse and human BMP4 are over 98% homologous and functionally equivalent (R&D Systems), however applicant has failed to disclose the relevant, identifying characteristics by their functional components, of all BMP4s, analogues, homologues and functional equivalents thereof, to sufficiently show the applicant was in possession of the entire scope of the claimed genus: BMP4. *See Eli Lilly,* 119F. 3d. at 1568, 43 USPQ2d at 1406.

Applicant's claim 10 is directed to a method for generating mesodermal cells from ES or EPL cells, comprising culturing ES cells or EPL cells in MEDII or its functional equivalent in order to generate embryoid bodies (EBMs); maintaining EBMs in culture for a time sufficient to allow aggregation of EBMs; transferring aggregated EBMs to gelatin-treated wells; allowing aggregated EBMs to adhere to said gelatin-treated wells; and culturing said adhered EBMs in serum free medium comprising BMP4 for a time sufficient to allow EBMs to generate mesodermal cells, and thereby generating mesodermal cells from ES cells or EPL cells. Claims 11-15 have the limitation of claim 10.

This claim is drawn to MEDII or its functional equivalent. MEDII is a specific chemically defined media, it requires conditioning by culturing HepG2 cells (ATCC HB-8065) in DMEM, supplemented with 10% FCS, 1mM L-glutamine, 40 mg/mL gentamycin, 0.1 mM B-mercaptoethanol and 1000 units LIF (See Specification, Pg 25 and Rathjen et al, 1999, Pg 603, col. 2). ES cells cultured in the presence of MEDII give rise to EPL cells, a distinct morphology not seen in presence of other media (See

Rathjen et al, 1999, Pg. 603, col. 2- Pg 604, col.1). Because MEDII is chemically defined, and elicits a specific response not replicated by other known media, it has not been made clear what constitutes a functional equivalent of MEDII. The specification fails to provide examples of functional equivalents, or teach the mechanism or pathway by which MEDII differentiates ES cells to EPL cells, or even the active ingredients that are responsible for the differentiation.

Claims 1-9 are also rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of directing a population of undifferentiated cells to differentiate along a mesodermal cell lineage, does not reasonably provide enablement for a method of directing any population of cells along mesodermal lineage. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

Only undifferentiated cells can be induced to differentiate, therefore the method is limited to stem cells and progenitor cells of mesodermal lineages, terminally differentiated cells cannot be induced to differentiate. Claims 2-9 have the limitations of claim 1, and are thus rejected on the same basis.

Claim Rejections – 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-15 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicant's claim 1 is directed to a method for directing a population of cells to differentiate along a mesodermal cell lineage, said method comprising culturing said cells in the presence of bone morphogenic protein 4 (BMP4) or a homologue, analogue or functional equivalent thereof for a time and

under conditions sufficient for said cells to preferentially differentiate into mesodermal cells or cells of a mesodermal lineage.

It is not clear what constitutes sufficient time and conditions for differentiation of cells into mesodermal cells or cells of a mesodermal lineage to occur. Furthermore, the term 'preferentially' makes it unclear if it is required that the cells differentiate into mesodermal cells or cells of a mesodermal lineage, or if it is only preferred. Still further, it is not clear what cells are of mesodermal lineage, it is not clear if they are part of the mesodermal layer of an adult body, or derived from primitive endoderm, which gives rise to the mesodermal layer. Claims 2-9 have the limitations of claim 1, and thus are rejected on the same basis.

Applicant's claim 10 is directed to a method for generating mesodermal cells from ES or EPL cells, comprising culturing ES cells or EPL cells in MEDII or its functional equivalent in order to generate embryoid bodies; maintaining EBMs in culture for a time sufficient to allow aggregation of said EBMs; transferring aggregated EBMs to gelatin-treated wells; allowing said aggregated EBMs to adhere to gelatin-treated wells; and culturing adhered EBMs in serum free medium comprising BMP4 for a time sufficient to allow EBMs to generate mesodermal cells, and thereby generate mesodermal cells from ES cells or EPL cells.

It is not clear how ES cells *or* EPL cells are cultured in MEDII; EPL cells are formed *by* culturing ES cells in MEDII (See Lake et al, Pg. 556, col. 1).

Claim Rejections – 35 USC § 102

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 3-5, & 7-9 are rejected under 35 U.S.C. 102(a) as being anticipated by Schuldiner et al (*PNAS,* 2000), in light of R&D Systems, in light of information from Dr. Benvenisty (of Schuldiner et al).

Applicant's claim 1 is directed to a method for directing a population of cells to differentiate along a mesodermal cell lineage, comprising culturing said cells in the presence of bone morphogenetic protein 4 (BMP4) or a homologue, analogue or functional equivalent thereof for a time and under conditions sufficient for said cells to preferentially differentiate into mesodermal cells or cells of a mesodermal lineage. Claim 3 requires the cells to be stem cells. Claim 4 requires the stem cells to be selected from the group consisting of embryonic stem cells, somatic stem cell, germ stem cells, adult renal stem cells, embryonic renal epithelial stem cells, embryonic endodermal stem cells, hepatocyte stem cells, mammary epithelial stem cells, bone marrow-derived stem cells, skeletal muscle stem cells, bone marrow mesenchymal stem cells, $CD34^+$ haematopoietic stem cells, and mesenchymal stem cells. Claim 7 requires the cells to be isolated from an animal selected from the group consisting of primates, livestock animals, laboratory test animals, companion animals, and avian species. Claim 8 requires the cells to be isolated from a mammal. Claim 9 requires the cells to be isolated from a human.

Schuldiner et al teach culturing human embryonic stem cells (H9 clone) in the presence of BMP4 (10 ng/ml) (See Pg 11307, col. 2- Pg 1308, col. 1) (Claims 3-4, & 7-9). The species origin of the BMP4 was not expressly stated in the paper, only that the BMP4 was obtained from R&D Systems, which sells Human BMP4 and Zebrafish BMP4; in response to a query regarding the type of BMP4 Dr. Benvenisky, of Schuldiner et al, disclosed the BMP4 used in the experiments to be Human BMP4 (See R&D Systems, and Communication from Dr. Benvenisty) Therefore human BMP4 was used to stimulate differentiation in human ES cells, See *In Re Baxter Travenol Labs,* 952 F.2d USPQ2d 1281 (Fed. Cir. 1991) (Claim 5). The ES cells differentiated into mesodermal cells, specifically chondrocytes and blood cells (See Pg 11311, col. 1 and Fig. 4) (Claim 1). Therefore the reference anticipates the claimed subject matter.

Claims 1, 3-4 & 7-8 are rejected under 35 U.S.C. 102(b) as being anticipated by Finley et al (*J Neurobiology,* 1999).

Applicant's claim 1 is directed to a method for directing a population of cells to differentiate along a mesodermal cell lineage, comprising culturing said cells in the presence of bone morphogenetic protein 4 (BMP4) or a homologue, analogue or functional equivalent thereof for a time and under conditions sufficient for said cells to preferentially differentiate into mesodermal cells or cells of a mesodermal lineage. Claim 3 requires the cells to be stem cells. Claim 4 requires the stem cells to be selected from the group consisting of embryonic stem cells, somatic stem cell, germ stem cells, adult renal stem cells, embryonic renal epithelial stem cells, embryonic endodermal stem cells, hepatocyte stem cells, mammary epithelial stem cells, bone marrow-derived stem cells, skeletal muscle stem cells, bone marrow mesenchymal stem cells, $CD34^+$ haematopoietic stem cells, and mesenchymal stem cells. Claim 7 requires the cells to be isolated from an animal selected from the group consisting of primates, livestock animals, laboratory test animals, companion animals, and avian species. Claim 8 requires the cells to be isolated from a mammal. Claim 9 requires the cells to be isolated from a human.

Finley et al teach culturing mouse embryonic stem cells (D3 ES line) in the presence of BMP4 (See Pg 273, col. 1-2) (Claims 3, 4, 7, & 8). The ES cells differentiated into cells of mesodermal lineage, evidenced by morphology, strong presence of vimentin (an intermediate filament protein expressed by early-migrating mesodermal and mesenchymal cells), and an increase in expression of *brachyury* (See Pg 278, col. 2- Pg. 281, col. 1) (Claim 1). They do not experiment with any other type of stem cell, such as those listed in claim 4, cells from animals listed in claim 7, nor do they use human cells (Claim 9). However, at the time the invention was made Finley et al taught the same steps performed by applicant, including use of the same cell line (D3 ES cell line) (See Pg 278, col. 2- Pg. 281, col. 1; See Specification, Pg. 25), but applicant extrapolates broader claims, including use of various types of stem cells, such as those listed in claim 4, cells from animals listed in claim 7, and use of human cells (Claim 9), without further experimental evidence. Therefore, applicant's actual induction of practice is the same as that taught by Finley et al, therefore Finley et al, was enabled for the use of all types of stem cells listed

in claim 4, all types of animals listed in claim 7, and humans (Claims 9). Thus, the reference anticipates the claimed subject matter.

Claims 1, 3-4, & 6-8 are rejected under 35 U.S.C. 102(b) as being anticipated by Johansson et al (*Mol. Cell. Biol.,* 1995), in light of Stem Cell Technologies, Inc.

Applicant's claim 1 is directed to a method for directing a population of cells to differentiate along a mesodermal cell lineage, comprising culturing said cells in the presence of bone morphogenetic protein 4 (BMP4) or a homologue, analogue or functional equivalent thereof for a time and under conditions sufficient for said cells to preferentially differentiate into mesodermal cells or cells of a mesodermal lineage. Claim 3 requires the cells to be stem cells. Claim 4 requires the stem cells to be selected from the group consisting of embryonic stem cells, somatic stem cell, germ stem cells, adult renal stem cells, embryonic renal epithelial stem cells, embryonic endodermal stem cells, hepatocyte stem cells, mammary epithelial stem cells, bone marrow-derived stem cells, skeletal muscle stem cells, bone marrow mesenchymal stem cells, $CD34^+$ haematopoietic stem cells, and mesenchymal stem cells. Claim 6 requires the BMP4 to be derived from a heterologous species to said cells. Claim 7 requires the cells to be isolated from an animal selected from the group consisting of primates, livestock animals, laboratory test animals, companion animals, and avian species. Claim 8 requires the cells to be isolated from a mammal.

Johansson et al teach culturing mouse embryonic stem cells from cell line CCE (129/Sv/Ev derived) (See Stem Cell Technologies, Inc) in the presence of human BMP4 (See Pg. 142, col. 1, Pg 145, col. 1-2, & Pg. 149, col. 1) (Claims 3, 4, & 6-8). The ES cells differentiated into mesodermal cells by day 5, as evidenced by increased expression of *brachyury*, spontaneous beating of cardiomyocytes, and presence of myeloid haematopoietic precursors (day 8) (See Pg. 145) (Claim 1). Therefore the reference anticipates the claimed subject matter.

Claim Rejections- 35 USC § 102/103

Claims 5 and 6 are rejected under 35 U.S.C. 102(b) as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Finley et al (*J Neurobiology*, 1999).

Applicant claims a method for directing a population of cells to differentiate along a mesodermal cell lineage, comprising culturing said cells in the presence of bone morphogenetic protein 4 (BMP4) or a homologue, analogue or functional equivalent thereof for a time and under conditions sufficient for said cells to preferentially differentiate into mesodermal cells or cells of a mesodermal lineage. Claim 5 requires the BMP4 to be derived from a homologous species to the cells. Claim 6 requires the BMP4 to be derived from a heterologous species from said cells.

Finley et al teach culturing mouse embryonic stem cells (D3 ES line) in the presence of BMP4 (See Pg 273, col. 1-2). The ES cells differentiated into cells of mesodermal lineage, evidenced by morphology, strong presence of vimentin (an intermediate filament protein expressed by early-migrating mesodermal and mesenchymal cells), and an increase in expression of *brachyury* (See Pg 278, col. 2- Pg. 281, col. 1).

Finley et al is silent on the species origin of the BMP4 used on the mouse ES cells. However, it appears the BMP4 used by applicant was Human BMP4, and it appears Human BMP4 was also used in the prior art. Therefore Finley et al teach culturing mouse ES cells in the presence of human BMP4, a species heterologous to the mouse ES cells (Claim 6).

If the BMP4 used by Finley et al was instead mouse BMP4, it would in fact be anticipatory of claim 5, which requires the BMP4 to be derived from a species homologous to the cells.

However, due to the 98% homology and known functional equivalency between mouse BMP4 and human BMP4, it would have been obvious to one of ordinary skill in the art at the time the invention was made to use mouse BMP4 or human BMP4 (Claims 5 & 6). One would have been motivated to use

mouse BMP4 in order to optimize experimental conditions, since mouse ES cells were used. One would have been motivated to use Human BMP4 because it is readily available from the popular supplier R&D Systems. One would have expected success using either mouse or human BMP4 because they are 98% homologous and R&D Systems teaches they can be used interchangeably, and Johansson et al has shown BMP4 does induce differentiation of ES cells into mesodermal lineages (See Pg 273, col. 1-2 and See Pg 278, col. 2- Pg. 281, col. 1).

Thus, the claimed invention as a whole was at least prima facie obvious, if not anticipated by the references, especially in the absence of evidence to the contrary.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-4, 7-10, & 13-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lake et al (*Journal of Cell Science*, 2000), in view of Schuldiner et al (*PNAS*, 2000), Finley et al (*J Neurobiology*, 1999), and Johansson et al (*Mol. Cell. Biol.*, 1995).

Applicant's claim 1 is directed to a method for directing a population of cells to differentiate along a mesodermal cell lineage, comprising culturing said cells in the presence of bone morphogenetic protein 4 (BMP4) or a homologue, analogue or functional equivalent thereof for a time and under conditions sufficient for said cells to preferentially differentiate into mesodermal cells or cells of a mesodermal lineage. Claim 3 requires the cells to be stem cells. Claim 4 requires the stem cells to be selected from the group consisting of embryonic stem cells, somatic stem cell, germ stem cells, adult renal stem cells, embryonic renal epithelial stem cells, embryonic endodermal stem cells, hepatocyte stem

cells, mammary epithelial stem cells, bone marrow-derived stem cells, skeletal muscle stem cells, bone marrow mesenchymal stem cells, $CD34^+$ haematopoietic stem cells, and mesenchymal stem cells. Claim 7 requires the cells to be isolated from an animal selected from the group consisting of primates, livestock animals, laboratory test animals, companion animals, and avian species. Claim 8 requires the cells to be isolated from a mammal. Claim 9 requires the cells to be isolated from a human.

Applicant's claim 10 is directed to a method for generating mesodermal cells from ES or EPL cells, comprising culturing ES cells or EPL cells in MEDII or its functional equivalent in order to generate embryoid bodies; maintaining EBMs in culture for a time sufficient to allow aggregation of said EBMs; transferring aggregated EBMs to gelatin-treated wells; allowing said aggregated EBMs to adhere to gelatin-treated wells; and culturing adhered EBMs in serum free medium comprising BMP4 for a time sufficient to allow EBMs to generate mesodermal cells, and thereby generate mesodermal cells from ES cells or EPL cells. Claim 13 requires the cells to be isolated from an animal selected from the group consisting of primates, livestock animals, laboratory test animals, companion animals, and avian species. Claim 14 requires the cells to be isolated from a mammal. Claim 15 requires the cells to be isolated from a human.

Lake et al teach culturing mouse ES cells (D3 line) in ES DMEM containing 50% MEDII, without LIF, to produce EPL cells. Embryoid bodies (what applicant refers to as EBMs, embryoid bodies produced in MEDII) were formed from a single EPL cell suspension plated at 1×10^5 cells/ml in bacteriological grade dishes in ES DMEM. With the exception of Lake et al using ES DMEM instead of DMEM conditioned with 50% MEDII during the aggregation of EPL cell embryoid bodies, and the transfer of aggregated EBs to gelatin-treated wells, the process by which Lake et al formed EPL cell EBs is identical to the process of forming EBMs in the current application (See Lake et al, Pg 556, col. 2; See Specification, Pg. 25-26). The culturing of EPL cell EBs in DMEM, as opposed to DMEM conditioned with 50% MEDII does not appear to have a significant effect on the cells; EPL cells were already formed,

and only reverted back to ES cells in the presence of LIF (See Lake et al, Pg 556, col.2). Therefore the continued use of DMEM conditioned with 50% MEDII during the aggregation of embryoid bodies appears to be an obvious design choice and does not have an affect on the morphology of the embryoid bodies formed. Similarly, transferring the EPL cell EBs to gelatin-treated wells is one method of culturing EBs; Lake et al chose to culture the EBs in a suspension culture. Both techniques are acceptable forms of culturing EBs, as neither shows any improvement or advantage over the other method, therefore the technique of culturing EBs is another an obvious design choice based on personal preference and material availability. Therefore Lake et al teach the same process of forming embryoid bodies as in the current application, and thus the EPL cell EBs formed by Lake et al are one and the same as the EBMs formed in the current application.

Lake et al continued to assess the EPL cell EBs' ability to differentiate by monitoring expression of *brachyury* and appearance of beating cardiocytes (which applicant calls cardiomyocytes) (See Lake et al, Pg 560). The EPL cell EBs did differentiate into mesodermal cells, as evidenced by 60% of cells showing beating muscle by day 6, and a 30-fold increase in expression of *brachyury* on days 2 and 3 (See Pg. 556, col. 2). However, others teach culturing EBs in the presence of BMP4 increases the degree of differentiation into mesodermal cells. Schuldiner et al teach culturing human EBs in presence of BMP4 induced differentiation into mesodermal cells, such as chondrocytes and blood cells (See Schuldiner et al, Pg. Pg 11307, col. 2- Pg 11308, col. 1; Pg 11311, col. 1; & Fig. 4). Finley et al teach treating aggregates (what applicant calls embryoid bodies) with BMP4 significantly reduced generation of neural and glial cells (ectodermal lineage) and increased generation of cells of mesodermal lineage, evidenced by morphology, presence of vimentin (an intermediate filament protein expressed by early-migrating mesodermal and mesenchymal cells), and an increase in expression of *brachyury* (See Finley et al, Pg. 273, col. 1; & Pg. 278, col. 2- Pg. 281, col. 1). Finally, Johansson et al teach ES cells cultured in chemically defined media form EBs and will exhibit low levels of spontaneous mesoderm formation, due

to factors synthesized by the ES/EBs themselves; however, EBs cultured in presence of BMP4 show increased differentiation into mesodermal cells, evidenced by enhancement in formation of cardiac muscle formation (See Johansson et al, Pg 147, col. 1- Pg 148, col. 2).

Therefore, it would have been obvious to the person of ordinary skill in the art at the time the invention was made to culture EPL cells EBs (what applicant calls EBMs) in the presence of BMP4 in order to generate cells of mesodermal lineage. The person of ordinary skill in the art would have been motivated to generate mesodermal cells by this method in order to increase control the differentiation pathway of the ES cells. One would expect success because Lake et al teach that EPL cells have a distinct morphology that predisposes them to formation of mesodermal lineages, and Schuldiner et al, Finley et al, and Johansson et al teach that directed differentiation towards mesoderm is increased when EBs are treated with BMP4; therefore treating cells predisposed to the mesodermal lineage with a growth factor that stimulates differentiation into the mesodermal lineage would be expected to produce mesodermal cells.

Therefore Lake et al, in view of Schuldiner et al, Finley et al, and Johansson et al teach a method of generating mesodermal cells by culturing D3 cells (mouse ES stems), in MEDII to form EPL cells, aggregating the EPL cells into EPL cell EBs (what applicant calls EBMs), culturing the EPL cell EBs (EBMs) in presence of BMP4 for a time sufficient to allow differentiation of EPL cell EBs into mesodermal cells (Claims 1-4, 7-8, 10, & 13-14).

Lake et al use mouse embryonic stem cells and EPL cells formed from the mouse ES cells (Claims 2, 3, 4, 7, & 8). They do not experiment with any other type of stem cell, such as those listed in claim 4, cells from animals listed in claims 7 and 13, nor do they use human cells (Claims 9 & 15). However, at the time the invention was made Lake et al, in view of Schuldiner et al, Finley et al, and Johansson et al, taught the same steps performed by applicant, including use of the same cell line (D3 ES cell line) (See Lake et al, Pg 556, col. 2; See Specification, Pg. 25), but applicant extrapolates broader

claims, including use of various types of stem cells, such as those listed in claim 4, cells from animals listed in claims 7 and 13, and use of human cells (Claims 9 & 15), without further experimental evidence. Therefore, applicant's actual induction of practice is the same as that taught by Lake et al, in view of Schuldiner et al, Finley et al, and Johansson et al, therefore Lake et al, in view of the others, was enabled for the use of all types of stem cells listed in claim 4, all types of animals listed in claim 7, and humans (Claims 9 & 15). And thus the invention as a whole would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Claim 5 is rejected under 35 U.S.C. 103(a) as obvious over Johansson et al (*Mol. Cell. Biol.*, 1995), in light of Stem Cell Technologies, Inc, further in light of R&D Systems.

Applicant claims a method for directing a population of cells to differentiate along a mesodermal cell lineage, comprising culturing said cells in the presence of bone morphogenetic protein 4 (BMP4) or a homologue, analogue or functional equivalent thereof for a time and under conditions sufficient for said cells to preferentially differentiate into mesodermal cells or cells of a mesodermal lineage. Claim 5 requires the BMP4 to be derived from a homologous species to the cells.

Johansson et al teach culturing mouse embryonic stem cells from cell line CCE (129/Sv/Ev derived) (See Stem Cell Technologies, Inc) in the presence of human BMP4 (See Pg. 142, col. 1, Pg 145, col. 1-2, & Pg. 149, col. 1). The ES cells differentiated into mesodermal cells by day 5, as evidenced by increased expression of *brachyury*, spontaneous beating of cardiomyocytes, and presence of myeloid haematopoietic precursors (day 8) (See Pg. 145).

Though Johansson et al teach using human BMP4 on mouse ES cells, it would have been obvious to one of ordinary skill in the art at the time the invention was made to use mouse BMP4 on mouse cells (Claim 5). One would have been motivated to use mouse BMP4 in order to optimize experimental conditions, using mouse BMP4 on mouse cells would better replicate the natural environment of the

developing cells, allowing one to more accurately access its affect on cell differentiation in vivo. One would have expected success using mouse BMP4 to induce mouse ES cells to differentiate into cells of a mesodermal lineage because success was obtained using human BMP4, which is 98% homologous and R&D Systems teaches they can be used interchangeably (See Johansson et al, Pg. 142, col. 1, Pg 145, col. 1-2, & Pg. 149, col. 1 & R&D Systems Technical Support). Therefore the invention as a whole would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Claim 6 is rejected under 35 U.S.C. 103(a) as obvious over Schuldiner et al (*PNAS,* 2000), in light of information from Dr. Benvenisty (of Schuldiner et al), and further in light of R&D Systems.

Applicant claims a method for directing a population of cells to differentiate along a mesodermal cell lineage, comprising culturing said cells in the presence of bone morphogenetic protein 4 (BMP4) or a homologue, analogue or functional equivalent thereof for a time and under conditions sufficient for said cells to preferentially differentiate into mesodermal cells or cells of a mesodermal lineage. Claim 6 requires the BMP4 to be derived from a heterologous species from said cells.

Schuldiner et al teach culturing human embryonic stem cells (H9 clone) in the presence of BMP4 (10 ng/ml) (See Pg 11307, col. 2- Pg 1308, col. 1) (Claims 3-4, & 7-9). The species origin of the BMP4 was not expressly stated in the paper, only that the BMP4 was obtained from R&D Systems, which sells Human BMP4 and Zebrafish BMP4; in response to a query regarding the type of BMP4 Dr. Benvenisky, of Schuldiner et al, disclosed the BMP4 used in the experiments to be Human BMP4 (See R&D Systems, and Communication from Dr. Benvenisty) Therefore human BMP4 was used to stimulate differentiation in human ES cells, See *In Re Baxter Travenol Labs,* 952 F.2d USPQ2d 1281 (Fed. Cir. 1991) (Claim 5). The ES cells differentiated into mesodermal cells, specifically chondrocytes and blood cells (See Pg 11311, col. 1 and Fig. 4) (Claim 1).

Though Schuldiner et al teach using human BMP4 on human ES cells, it would have been obvious to one of ordinary skill in the art at the time the invention was made to use mouse BMP4 on mouse cells (Claim 5). One would have been motivated to use mouse BMP4 instead of human BMP4 if they had mouse BMP4 readily available in their lab. One would have expected success using mouse BMP4 to induce human ES cells to differentiate into cells of a mesodermal lineage because success was obtained using human BMP4, mouse BMP4 and Human BMP4 are 98% homologous, and R&D Systems teaches they can be used interchangeably (See Pg 11307, col. 2- Pg 1308, col. 1 & R&D Systems Technical Support). Therefore the invention as a whole would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Allison M Ford whose telephone number is 571-272-2936. The examiner can normally be reached on M-F 7:30-5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Wityshyn can be reached on 571-272-0927. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

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